PATENT COOPERATION TREATY

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From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

To:
TESTA, HURWITZ & THIBEAULT, LLP
Attn. Steel, Diana M.
High Street Tower
125 High Street
Boston, MA 02110
UNITED STATES OF AMERICA

Date of mailing *(day/month/year)*	04/01/2005
Applicant's or agent's file reference: RBN-003PC	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No.: PCT/US2004/010686	International filing date *(day/month/year)*: 07/04/2004
Applicant: RIBONOMICS INC.	

RECEIVED
JAN 11 2005
TESTA, HURWITZ & THIBEAULT, LLP
PATENT DEPARTMENT

1. [X] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

   When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Fascimile No.: (41-22) 740.14.35

   For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   [ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   [ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until **30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stefanie Büchler

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the *PCT Applicant's Guide*, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report and the written opinion of the International Searching Authority, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims,description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only (see *PCT Applicant's Guide*, Annexes B1 and B2).

The attention of the applicant is drawn to the fact that amendments to the claims under Article 19 are not allowed where the International Searching Authority has declared, under Article 17(2), that no international search report would be established (see *PCT Applicant's Guide*, Volume I/A, paragraph 296).

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

If a demand for international preliminary examination is made, the written opinion of the International Searching Authority will, except in certain cases where the International Preliminary Examining Authority did not act as International Searching Authority and where it has notified the International Bureau under Rule 66.1*bis*(b), be considered to be a written opinion of the International Preliminary Examining Authority. If a demand is made, the applicant may submit to the International Preliminary Examining Authority a reply to the written opinion together, where appropriate, with amendments before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later (Rule 43*bis*.1(c)).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see the *PCT Applicant's Guide,* Volume II.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
RBN-003PC		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2004/010686	07/04/2004	07/04/2003
Applicant		
RIBONOMICS INC.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 10 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
      [ ] The international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   b. [X] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. [ ] **Certain claims were found unsearchable** (See Box II).

3. [X] **Unity of invention is lacking** (see Box III).

4. With regard to the **title,**
   [X] the text is approved as submitted by the applicant.
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   [X] the text is approved as submitted by the applicant.
   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regards to the **drawings,**
   a. the figure of the **drawings** to be published with the abstract is Figure No. _____
      [ ] as suggested by the applicant.
      [ ] as selected by this Authority, because the applicant failed to suggest a figure.
      [ ] as selected by this Authority, because this figure better characterizes the invention.
   b. [X] none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/010686

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. type of material

   [X] a sequence listing

   [ ] table(s) related to the sequence listing

   b. format of material

   [X] in written format

   [X] in computer readable form

   c. time of filing/furnishing

   [ ] contained in the international application as filed

   [ ] filed together with the international application in computer readable form

   [X] furnished subsequently to this Authority for the purpose of search

2. [X] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-20,25-30,37

   screening methods involving the comparison of RNA or protein levels of at least one component of an isolated mRNP from two different cellular phenotypes or states (e.g. treated vs. untreated)

   ---

2. claims: 21-24

   method for identifiying a gene or gene product involved in a physiological pathway by isolating additional components of an mRNP complex that contains a component already known to be involved in said pathway

   ---

3. claims: 31,32

   method for identifying an insulin production regulating protein agent characterized by its ability to bind to the 3' or 5' untranslated region of a preproinsulin mRNA

   ---

4. claim: 33

   mRNP complex involved in glucose or lipid metabolism which comprises PTB protein and an mRNA associated with PTB

   ---

5. claims: 34-36

   method for identifying a component of an mRNP complex by expression profiling of RNA with or without prior inhibition of expression of an RNA binding protein

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 . G01N33/68 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PHELPS W. B.: "Innovative Systems Biology"'Online! 6 November 2002 (2002-11-06), XP002291620 Retrieved from the Internet: URL:http://www.ribonomics.com/news/presentations/ribonomics_RNA_in_Drug_Development.pdf> 'retrieved on 2004-08-05!	1-11,14, 15, 18-20, 30,37
Y	the whole document	12
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 October 2004	04.01.05
Name and mailing address of the ISA	**Authorized officer**
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Rutz, B

2

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category [d]	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TILLMAR LINDA ET AL: "Hypoxia may increase rat insulin mRNA levels by promoting binding of the polypyrimidine tract-binding protein (PTB) to the pyrimidine-rich insulin mRNA 3'-untranslated region." MOLECULAR MEDICINE (CAMBRIDGE, MASS.) MAY 2002, vol. 8, no. 5, May 2002 (2002-05), pages 263-272, XP002291619 ISSN: 1076-1551	31-33
Y	the whole document	12
X	CHEATHAM B. ET AL.: "A ribonomic analysis of adipocytes: a systems biology tool"'Online! 2 December 2002 (2002-12-02), XP002291621 Retrieved from the Internet: URL:http://www.ribonomics.com/news/presentations/ribonomics_MetabolicDisease2002Poster.pdf> 'retrieved on 2004-08-05! the whole document	1-11,14, 15, 18-20, 25-30,37
X	US 2002/004211 A1 (TENENBAUM SCOTT A ET AL) 10 January 2002 (2002-01-10) paragraph '0004!; figures 2,4,8; table 1 paragraph '0019! paragraph '0049! paragraph '0064! paragraph '0072! - paragraph '0074!	1,6,7, 9-12,14, 20-29
X	CEMAN S ET AL: "Isolation of an FMRP-associated messenger ribonucleoprotein particle and identification of nucleolin and the fragile X-related proteins as components of the complex." MOLECULAR AND CELLULAR BIOLOGY. DEC 1999, vol. 19, no. 12, December 1999 (1999-12), pages 7925-7932, XP002302896 ISSN: 0270-7306 the whole document	21-24

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OHASHI SACHIYO ET AL: "Identification of mRNA/protein (mRNP) complexes containing Puralpha, mStaufen, fragile X protein, and myosin Va and their association with rough endoplasmic reticulum equipped with a kinesin motor." THE JOURNAL OF BIOLOGICAL CHEMISTRY. 4 OCT 2002, vol. 277, no. 40, 4 October 2002 (2002-10-04), pages 37804-37810, XP002302897 ISSN: 0021-9258 the whole document	21-24
X	TENENBAUM SCOTT A ET AL: "Ribonomics: Identifying mRNA subsets in mRNP complexes using antibodies to RNA-binding proteins and genomic arrays" METHODS (ORLANDO), vol. 26, no. 2, February 2002 (2002-02), pages 191-198, XP002291623 ISSN: 1046-2023 page 194, right-hand column, line 12 - page 195, left-hand column, paragraph 1	21-24
X	GAVIN A-C ET AL: "Functional organization of the yeast proteome by systematic analysis of protein complexes" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 415, January 2002 (2002-01), pages 141-147, XP002958851 ISSN: 0028-0836 page 143, right-hand column, last paragraph - page 144, left-hand column, paragraph 1; figure 3	21-24
X	TILLMAR LINDA ET AL: "Control of insulin mRNA stability in rat pancreatic islets. Regulatory role of a 3'-untranslated region pyrimidine-rich sequence." THE JOURNAL OF BIOLOGICAL CHEMISTRY. 11 JAN 2002, vol. 277, no. 2, 11 January 2002 (2002-01-11), pages 1099-1106, XP002302898 ISSN: 0021-9258	31-33
Y	the whole document	35,36

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2

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HIERONYMUS HALEY ET AL: "Genome-wide analysis of RNA-protein interactions illustrates specificity of the mRNA export machinery." NATURE GENETICS. FEB 2003, vol. 33, no. 2, February 2003 (2003-02), pages 155-161, XP002302899 ISSN: 1061-4036	34
Y	the whole document	35,36

X	LELIVELT M J ET AL: "Yeast Upf proteins required for RNA surveillance affect global expression of the yeast transcriptome" MOLECULAR AND CELLULAR BIOLOGY, AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, US, vol. 19, no. 10, October 1999 (1999-10), pages 6710-6719, XP002977598 ISSN: 0270-7306 the whole document	34

A	RIBONOMICS INC.: "Research & Technology"'Online! 17 March 2003 (2003-03-17), XP002291622 Retrieved from the Internet: URL:http://web.archive.org/web/20030317064208/http://www.ribonomics.com/technology/index.html> 'retrieved on 2004-08-05!	

A	TENENBAUM S A: "IDENTIFYING MRNA SUBSETS IN MESSENGER RIBONUCLEOPROTEIN COMPLEXES BY USING CDNA ARRAYS" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 97, no. 26, 19 December 2000 (2000-12-19), pages 14085-14090, XP000995310 ISSN: 0027-8424	

A	KEENE JACK D: "Ribonucleoprotein infrastructure regulating the flow of genetic information between the genome and the proteome" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 98, no. 13, 19 June 2001 (2001-06-19), pages 7018-7024, XP002291624 ISSN: 0027-8424	

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2

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KEENE JACK D ET AL: "Eukaryotic mRNPs may represent posttranscriptional operons" MOLECULAR CELL, vol. 9, no. 6, June 2002 (2002-06), pages 1161-1167, XP002291625 ISSN: 1097-2765	
A	RODGERS NANCY D ET AL: "Identifying mRNAs bound by RNA-binding proteins using affinity purification and differential display." METHODS (SAN DIEGO, CALIF.) FEB 2002, vol. 26, no. 2, February 2002 (2002-02), pages 115-122, XP002291626 ISSN: 1046-2023	
A	BROWN V ET AL: "Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in fragile X syndrome." CELL. 16 NOV 2001, vol. 107, no. 4, 16 November 2001 (2001-11-16), pages 477-487, XP002291627 ISSN: 0092-8674	
P,X	KNOCH KLAUS-PETER ET AL: "Polypyrimidine tract-binding protein promotes insulin secretory granule biogenesis." NATURE CELL BIOLOGY. MAR 2004, vol. 6, no. 3, March 2004 (2004-03), pages 207-214, XP002302900 ISSN: 1465-7392 the whole document	31,33
P,X	HEROLD ANDREA ET AL: "Genome-wide analysis of nuclear mRNA export pathways in Drosophila." THE EMBO JOURNAL. 15 MAY 2003, vol. 22, no. 10, 15 May 2003 (2003-05-15), pages 2472-2483, XP002302901 ISSN: 0261-4189 the whole document	34

2

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2002004211 A1	10-01-2002	US 2003235830 A1	25-12-2003
		US 2003211466 A1	13-11-2003
		US 2004096878 A1	20-05-2004
		AU 2743101 A	09-07-2001
		CA 2396058 A1	05-07-2001
		EP 1254370 A1	06-11-2002
		JP 2004520002 T	08-07-2004
		WO 0148480 A1	05-07-2001

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2004/010686	07.04.2004	07.04.2003

International Patent Classification (IPC) or both national classification and IPC
G01N33/68, G01N33/53

Applicant
RIBONOMICS INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Rutz, B

Telephone No. +49 89 2399-7828

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☐ The following document has not been furnished:

   ☐ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. ☒ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

4. Additional observations, if necessary:

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos.

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	13,16,17,23,24,32-36
	No:	Claims	1-12,14,15,18-22,25-31,37
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	13,16,17,23,24,32-36
Industrial applicability (IA)	Yes:	Claims	1-37
	No:	Claims	-

2. Citations and explanations

see separate sheet

Re Item IV.

The separate inventions/groups of inventions are:

Claims 1-20, 25-30, 37:
screening methods involving the comparison of RNA or protein levels of at least one component of an isolated mRNP from two different cellular phenotypes or states (e.g. treated vs. untreated)
Claims 21-24:
method for identifiying a gene or gene product involved in a physiological pathway by isolating additional components of an mRNP complex that contains a component already known to be involved in said pathway
Claims 31, 32:
method for identifying an insulin production regulating protein agent characterized by its ability to bind to the 3' or 5' untranslated region of a preproinsulin mRNA
Claim 33:
mRNP complex involved in glucose or lipid metabolism which comprises PTB protein and an mRNA associated with PTB
Claims 34-36:
method for identifying a component of an mRNP complex by expression profiling of RNA with or without prior inhibition of expression of an RNA binding protein

They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons:

Rule 13.1 PCT states that for unity of invention to be present, all subject-matter should be linked by a single general inventive concept. The common concept (technical relationship) linking the above identified groups together is mRNP complexes. However, this concept cannot be regarded as the "single general inventive concept" required by Rule 13.2 PCT because it is neither novel nor inventive (see for example US2002/0004211).

Re Item V.

1. The following documents are referred to in this communication:

D1 : PHELPS W. B.: "Innovative Systems Biology"[Online] 6 November 2002 (2002-11-06), XP002291620 Retrieved from the Internet: URL:http://www.ribonomics.com/news/presentations/ribonomics_RNA_in_Drug_Development. pdf>; [retrieved on 2004-08-05]

D2: TILLMAR LINDA ET AL: "Hypoxia may increase rat insulin mRNA levels by promoting binding of the polypyrimidine tract-binding protein (PTB) to the pyrimidine-rich insulin mRNA 3'-untranslated region." MOLECULAR MEDICINE (CAMBRIDGE, MASS.) MAY 2002, vol. 8, no. 5, May 2002 (2002-05), pages 263-272, XP002291619 ISSN: 1076-1551

D3 : CHEATHAM B. ET AL.: "A ribonomic analysis of adipocytes: a systems biology tool"[Online] 2 December 2002 (2002-12-02), - 3 December 2002 (2002-12-03) XP002291621 Retrieved from the Internet: URL:http://www.ribonomics.com/news/presentations/ribonomics_MetabolicDisease2002Post er.pdf>; [retrieved on 2004-08-05]

D4 : US 2002/004211 A1 (TENENBAUM SCOTT A ET AL) 10 January 2002 (2002-01-10)

D5: CEMAN S ET AL: "Isolation of an FMRP-associated messenger ribonucleoprotein particle and identification of nucleolin and the fragile X-related proteins as components of the complex." MOLECULAR AND CELLULAR BIOLOGY. DEC 1999, vol. 19, no. 12, December 1999 (1999-12), pages 7925-7932, XP002302896 ISSN: 0270-7306

D6: OHASHI SACHIYO ET AL: "Identification of mRNA/protein (mRNP) complexes containing Puralpha, mStaufen, fragile X protein, and myosin Va and their association with rough endoplasmic reticulum equipped with a kinesin motor." THE JOURNAL OF BIOLOGICAL CHEMISTRY. 4 OCT 2002, vol. 277, no. 40, 4 October 2002 (2002-10-04), pages 37804-37810, XP002302897 ISSN: 0021-9258

D7: TENENBAUM SCOTT A ET AL: "Ribonomics: Identifying mRNA subsets in mRNP complexes using antibodies to RNA-binding proteins and genomic arrays" METHODS (ORLANDO), vol. 26, no. 2, February 2002 (2002-02), pages 191-198, XP002291623 ISSN: 1046-2023

D8: GAVIN A-C ET AL: "Functional organization of the yeast proteome by systematic analysis of protein complexes" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 415, January 2002 (2002-01), pages

141-147, XP002958851 ISSN: 0028-0836

D9: TILLMAR LINDA ET AL: "Control of insulin mRNA stability in rat pancreatic islets. Regulatory role of a 3'-untranslated region pyrimidine-rich sequence." THE JOURNAL OF BIOLOGICAL CHEMISTRY. 11 JAN 2002, vol. 277, no. 2, 11 January 2002 (2002-01-11), pages 1099-1106, XP002302898 ISSN: 0021-9258

D10: HIERONYMUS HALEY ET AL: "Genome-wide analysis of RNA-protein interactions illustrates specificity of the mRNA export machinery." NATURE GENETICS. FEB 2003, vol. 33, no. 2, February 2003 (2003-02), pages 155-161, XP002302899 ISSN: 1061-4036

D11: LELIVELT M J ET AL: "Yeast Upf proteins required for RNA surveillance affect global expression of the yeast transcriptome" MOLECULAR AND CELLULAR BIOLOGY, AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, US, vol. 19, no. 10, October 1999 (1999-10), pages 6710-6719, XP002977598 ISSN: 0270-7306

2. Novelty (Art. 33(2) PCT)

2.1. The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claim 1 is not new in the sense of Article 33(2) PCT.
Document D1 discloses: A method for the identification of therapeutic targets which is based on the comparison of mRNP composition in different cellular states (e.g. diseased vs. healthy, treated vs. untreated etc.). As in present application this method is termed ribonomics. It describes the generation of a list of RNA binding proteins (RBPs) and its use in the screening method. Furthermore, D1 shows a list of gene classes to be found in a typical ribonomics experiment ("Simplifiying gene expression data"). The classes mentioned (e.g. "kinases, phosphatases, proteases, receptors ...") are largely identical with the classes mentioned in claim 15 of present application.
An example given in D1 relates to the study of obesity and involves RBP expression analysis in pre-adipocyte and adipocyte cells and the comparison between lean and obese humans. The effect of insulin and the β3 agonist BRL-37344 on RBP expression levels in adipocytes are tested.

Claims 1-11, 14, 15, 18-20, 25-30 and 37 lack novelty over D1 (Art. 33(2) PCT).

2.2. The same arguments apply vis à vis D3 which also shows the identification of therapeutic targets by ribonomic analysis.

Claims 1-11, 14, 18-20, 25-30 and 37 lack novelty over D3 (Art. 33(2) PCT).

2.3. Document D4 discloses the general strategy of ribonomics, but contains no reference to the specific application of this technology to glucose or lipid metabolism. Furthermore, D4 discloses the use of PTB as a RNA binding protein in the described methods (Table 1).

Claims 1, 6, 9-12, 14, 20, 25-28 and 37 lack novelty over D4 (Art. 33(2) PCT).

2.4. Documents D5-D8 all disclose the isolation of RNP complexes which contain at least one known compound and identify further compounds of said complex by different methods (see for example D8, Fig. 3).

Claims 21 and 22 lack novelty over any one of D5-D8 (Art. 33(2) PCT).

2.5. D9 discloses a method for identifying a protein (probably PTB) by probing for specific binding to the pyrimidine-rich insulin mRNA 3'-UTR sequence (p. 1101, right column, last paragraph). Furthermore, it describes the importance of said binding for glucose metabolism (p. 1105, left column, paragraph 3).

Claim 31 lacks novelty over D9 (Art. 33(2) PCT).

3. Inventive Step (Art. 33(3) PCT)

3.1. Claim 13 lacks inventive step over D1 because said document discloses a straightforward way to identify additional mRNA binding proteins by bioinformatic methods (e.g. search for RNA binding motifs) for use in the described method ("Overview of RNA Binding Proteins", "RBP Gene List Fabrication"). As an example of an RBP the HuB protein is mentioned. The list of RBPs disclosed in Fig. 10-22 can therefore only be considered an obvious selection.

Claim 13 lacks inventive step over D1 (Art. 33(2) PCT).

3.2. Claims 16 and 17 which are dependent on claim 1 lack inventive step because they contain a list of therapeutic targets which are obvious for at least some of the methods falling under the scope of claim 1. Similar genes have been identified in D1 for certain conditions (e.g. CACNB3, CELSR and MBNL during neuronal and muscle differentiation in G11 cells). Most of the genes listed in claims 16 and 17 are known to be involved in signaling pathways and regulatory circuits of mammalian cells (e.g. kinases, phosphatases, receptors, see Table in D1: "Genes upregulated in RNP w/o

change in Totals"). It was therefore obvious for the skilled person that those genes would be differentially regulated under certain conditions.

Claims 16 and 17 lack inventive step over D1 (Art. 33(2) PCT).

3.3. Claims 23 and 24 relate to well known methods in the field of functional genomics the use of which to verify the function of a gene (i.e. loss of function or knock-down experiments) would have been obvious to the skilled person.

Claims 23 and 24 are not inventive over any one of D5-D8 (Art. 33(3) PCT).

3.4. Both documents, D2 and D9, disclose the binding site for PTB on the rat insulin II mRNA (D2, Table 1, Ins-PRS wild-type; D9, Fig. 1 B). The corresponding sequences are almost identical to the second sequence listed in claim 32. Furthermore, both documents point to the existence of mRNP complexes comprising PTB and preproinsulin mRNA (D2, Fig. 6; D9, p. 1105, left column, paragraphs 2 and 3).

Claims 32 and 33 lack inventive step over either one of D2 or D9 (Art. 33(3) PCT).

3.5. Both documents, D10 and D11, disclose methods for identifying mRNAs which show altered expression levels in response to a loss of function of RNA binding proteins. At least D11, in addition, describes a method to inactivate the genes (upf1-3) by transformation with a nucleic acid (p. 6711, left column, first paragraph). The use of said method to investigate mRNP complexes involved in glucose or lipid metabolism (as described, for example, in D2 or D9) appears obvious to the skilled person.

Claims 34-36 are not inventive over the combination of D10 or D11 with D2 or D9 (Art. 33(3) PCT).